



International Journal of Medical Research & Health Sciences

www.ijmrhs.com

Volume 4 Issue 1

Coden: IJMRHS

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ISSN: 2319-5886

Received: 23rd Aug 2014

Revised: 10th Sep 2014

Accepted: 16th Nov 2014

Research article

STUDY ON ASSOCIATION OF SERUM HOMOCYSTEINE WITH CORONARY HEART DISEASE IN RURAL POPULATION

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ABSTRACT

Background: CAD is the major cause of death. Many factors are responsible for causing CAD, but in 5 to 10 percent of CAD patients have none of the known risk factors. Risk factor modification is an integral part of the management of patients who have or are at risk for cardiovascular disease. Clinicians who care for patients with cardiovascular disease should be aware of new risk factors. Significant associations exist between established and new risk factors, and better understanding of new risk factors may shed light on the pathogenetic mechanisms of established risk factors. **Objectives:** To study the association of homocysteine in patients with coronary heart disease. **Methods:** This study was conducted in 50 patients of CAD and 50 people as a control group. All patients underwent a standard clinical examination and a blood draw for a lipid profile and total fasting serum homocysteine assay. Pearson chi-square test was used to assess the statistical significance. P value of less than 0.01 indicates highly significant and value of less than 0.05 indicates significant. **Results:** The cutoff value of homocysteine used in this study was 17micro mol/L. In case group 43 patients (86%) were showed raised homocysteine, and in control group 12 patients (24%) were showed raised homocysteine. And here the p value is <0.001 with the relative risk of 19.45. It shows raised homocysteine is statistically highly significant. **Conclusions:** The association of hyperhomocysteinemia with CHD was significant. Homocysteine values were higher in smokers and hypertensives.

Keywords: Coronary heart disease, Homocysteine,

INTRODUCTION

Coronary artery disease (CAD) is the major cause of death in the world today.¹ Many factors are responsible for causing CAD, but some patients have none of the known major risk factors. Some of the risk factors for CAD such as age, sex and family background cannot be altered, but others such as arterial hypertension, diabetes, smoking and hyperlipidemia could be controlled. It is notable that 5 to 10 percent of CAD patients have none of the known risk factors.² Common symptoms of CAD are

angina, dyspnoea, palpitation and profuse sweating. Regarding physical examination in CAD patients with diabetes and/or peripheral arterial disease, clinicians should search for evidence of atherosclerotic disease at other sites, such as an abdominal aortic aneurysm, carotid arterial bruits, and diminished arterial pulses in the lower extremities. Risk factor modification is an integral part of the management of patients who have or are at risk for cardiovascular disease. In addition to

established cardiovascular risk factors, clinical research has identified more than 100 other conditions that may be associated with an increased risk for cardiovascular disease. Clinicians who care for patients with cardiovascular disease should be aware of new risk factors. Almost 25% of patients with premature cardiovascular disease do not have any established risk factors.³ As a result of reductions in morbidity and mortality attributable to hypertension, smoking, and dyslipidemia^{4,5}, the relative contribution of new risk factors to the total burden of cardiovascular disease is likely to increase. Significant associations exist between established and new risk factors, and better understanding of new risk factors may shed light on the pathogenetic mechanisms of established risk factors. On the basis of a growing body of evidence, the 1996 Bethesda Conference acknowledged left ventricular hypertrophy, hyperhomocysteinemia, lipoprotein (a) excess, hypertriglyceridemia, hyperfibrinogenemia (among other thrombogenic factors), and oxidative stress as possible risk factors for CAD. The serum concentration of the amino acid homocysteine is positively associated with the risk of ischemic heart disease, deep vein thrombosis and pulmonary embolism, and stroke.⁶ There is uncertainty over whether these associations are causal.⁷ Resolving the question of causality is important because serum homocysteine can be lowered by the B vitamin folic acid,⁸ raising the prospect of a simple and safe means of prevention. In order to consider homocysteine a causative rather than coincidental factor, plausible mechanisms for homocysteine action must be tested. The most common and plausible mechanism are oxidative damage and vascular smooth muscle cell proliferation. Much of the endothelial dysfunction attributed to homocysteine is thought to occur primarily from oxidative stress.⁹ This is also one of the proposed mechanisms for DNA damage and carcinogenesis. In numerous in vitro studies, homocysteine was able to trigger proliferation of vascular smooth muscle cells¹⁰ an effect which is attenuated by folic acid. By increasing vascular smooth muscle proliferation, the arterial lumen space will be narrower, typically considered to be deleterious for coronary artery disease. The normal reference range for plasma total homocysteine is usually defined as the 2.5th to 97.5th percentile

interval for presumably healthy people. The lower limit is typically 5 $\mu\text{mol/L}$, but the upper limit varies considerably among clinical laboratories. Furthermore, in different populations, the upper limit may vary between 10 and 20 $\mu\text{mol/L}$, depending on age (levels increase with age), sex (levels are higher in men than in women), ethnic group, and dietary intake of folate. Rather than defining a level of homocysteine as either normal or abnormal, it may be more useful to consider homocysteine, like cholesterol and C-reactive protein, as a graded risk factor for cardiovascular disease.¹¹

Aim of the study

1. To study the association of homocysteine in patients with coronary heart disease.
2. To study the role of raised homocysteine levels as risk factors when compared to other known risk factors in coronary heart disease.

MATERIALS AND METHODS

After getting Ethical committee clearance, this study was conducted in 50 patients with coronary heart disease above 16 years of age, of both sex and also included 50 age and sex matched people as a control group. Informed consent was obtained from all the participants. This study was conducted in the department of medicine, Meenakshi medical college hospital and research institution, Kanchipuram during July 2013 to January 2014.

Study design: Case control study.

Exclusion criteria : Patients with Renal impairment, Pregnancy, Hypothyroidism, Nephrotic syndrome, cancer and Patients on Drugs like Sodium Valproate, Carbamazepine, Cyclosporin, Methotrexate, Theophylline, Levodopa, Metformin, Estrogen (OCP), INH, Fibrates and Niacin were excluded.

All patients (cases and controls) underwent a standard clinical examination by nurses and physicians, which included anthropometry (height, weight, waist-hip ratio), blood pressure, and a blood drawn after six weeks of acute coronary event, for Basic Biochemical Analyses and Fasting total cholesterol, HDL-C, LDL-C, and triglycerides and tHcy. Patients also received dietary and smoking counselling when necessary. Individuals also completed a questionnaire that incorporated numerous risk related issues, including a history of hypertension, family history, cholesterol medication use, and diabetes (ever treated or

diagnosed by a physician), and, in women, menopausal status and use of hormones. Patients were classified as either never- or ever-smokers. Hypertension was defined as a blood pressure above 140/90 mm Hg, a history of hypertension, or the use of antihypertensive medications. Diabetes mellitus was diagnosed if the patient was using insulin or an oral hypoglycemic agent or reported a history of diabetes mellitus.

tHcy Assay : Total fasting serum tHcy was measured on samples drawn on follow up visit. Serum tHcy has been shown to be '10% to 30% higher than plasma tHcy. A tHcy cut off point of 17 micro mol/L was used for all initial interaction analyses, which is the 90th percentile of tHcy values obtained in the lab. The plasma homocysteine levels were calculated by using the (Bio-Rad kit) homocysteine microplate enzyme immunoassay.¹² This is intended for the quantitative determination of L-homocysteine in human serum or plasma.

Protocol: Venous blood samples were obtained from a study population by trained medical or senior nursing staff from antecubital vein. Blood was transferred into containers containing EDTA for homocysteine and lipoprotein assay. Within 15 mins of collection, platelet poor plasma was obtained by centrifugation at room temperature for 15 mins at 3000 rpm and then transferred to a - 80°C freezer. Blind analysis of all samples was performed in batches at completion of sample collection.

Statistical analysis: The comparison of risk factors in case and control group was done by the t- test for equality of means. All values were calculated as mean \pm standard deviation. Pearson chi-square test was used to assess the statistical significance. P value of less than 0.01 indicates highly significant and value of less than 0.05 indicates significant. The odds ratio is used to estimate the relative risk.

RESULTS

The case group comprised patients with age ranging from 34 to 85, and a mean age of 55.96. In the control group age ranged from 34 to 70, with a mean age of 51.50. A male preponderance of 70% was seen in the case group, but was not of statistical significance. The cutoff value of homocysteine used in this study was 17micro mol/L. In case group 43 patients (86%) were showed raised homocysteine, and in control

group 12 patients (24%) were showed raised homocysteine. Pearson chi-square test was used to assess the statistical significance. And here the p value is <0.001 with the relative risk of 19.45. It shows raised homocysteine is statistically highly significant. LDL-C value of more than 130 mg/dl was observed in 18 patients (36%) when compared to 1 (2%) in the control group. And value of 100 to 129 mg/dl was observed in 23(46%) when compared to 31(62%) in control group. Value of 90 to 99 mg/dl was observed in 3(6%) when compared to 12(24%) in control group. Value of less than 90 mg/dl was observed in 6(12%) when compared to 6(12%) in control group. Mean value of LDL-C is 122.64 in case group and 106.48 in control group. T-test for equality means shows p-value of <0.001 and is highly significant. HDL-C value of less than 40 mg/dl was observed in 28 patients (56%) in case group when compared to 35 (70%) in the control group. And value of more than 40 mg/dl was observed in 22(44%) when compared to 15(30%) in control group. Mean value of HDL-C is 39.10 in case group and 38.88 in control group. T-test for equality means shows p-value of 0.842 and is not significant. The mean value of total cholesterol in case group is 160.34 mg/dl and 171.98 in control group. And the association in between these two is not statistically significant. The mean value of triglyceride in case group is 115.52 mg/dl and 106.50 in control group. And the association in between these two is not significant. Family history of premature CHD was present in 16 patients in case group and 5 in control group. Pearson chi-square test shows p-value of 0.007 and the association is statistically significant. Sedentary lifestyle was present in 25 patients in case group and 12 in control group. P-value is 0.007 and the association is statistically significant. In case group 19 patients (38%) were alcoholics when compared to 12 (24%) in the control group. p-value is 0.130 and the association is not statistically significant. Diabetes was present in 22 (44%) patient in case group and 8(16%) in control group. p-value is 0.002 and the association is statistically significant. In case group 22 patients (44%) were smokers when compared to 13 (26%) in the control group. p-value is 0.059 and the association is not statistically significant. Hypertension was present in 26 (52%) patient in case group and 5(10%) in control group. p-

value is < 0.001 and the association is highly significant. In case group 52% of patients were hypertensive and in that hypertension population 86% have raised homocysteine and the association of raised homocysteine and hypertension is statistically significant with P-value was 0.023. In case group 44% of patients were smokers and in that smoker population 86% have raised homocysteine. P-value was 0.017 and the association of raised homocysteine and smoking is statistically significant.

Age, lifestyle, Family history of premature CHD, and parameters like BMI and waist to hip ratio were not statistically associated with levels of homocysteine.

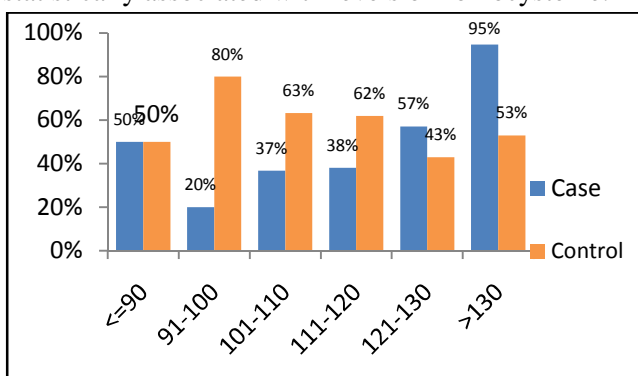


Fig: 1 Comparison of LDL-C between Case and Control Group

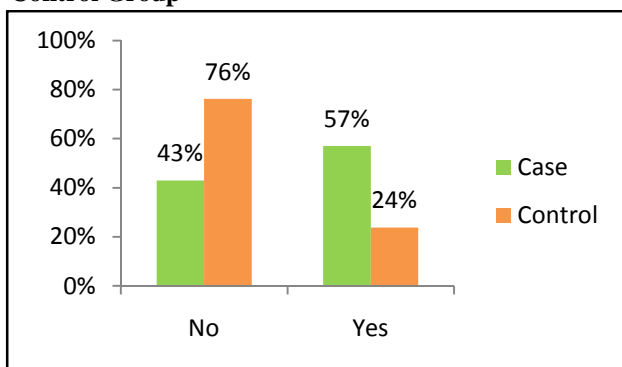


Fig: 2 Comparison of Family History of premature CHD between Case and Control Group

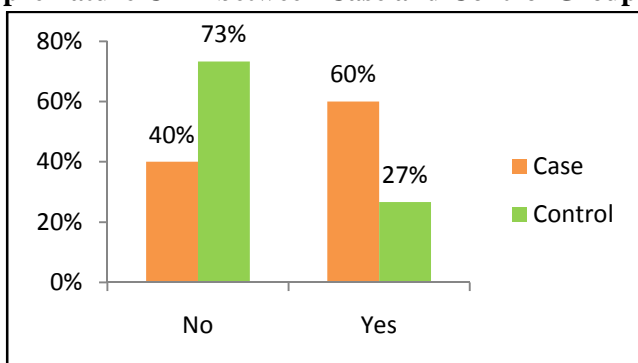


Fig: 3 Comparison of presence of Diabetes between Case and Control Group

Table: 1 Comparison of Homocysteine between Case and Control Group

		Group		
		Case	Control	Total
HCY <17	Count	7	35	45
	% with In HCY	15.6	84.4	100
	% with In GROU	14	76	45
HCY >17	Count	43	12	55
	% with In HCY	78.2	21.8	100
	% with In GROU	86	24	55
Total	Count	50	50	100
	% with In HCY	50	50	100
	% with In GROU	100	100	100
		Value	df	P value
Pearson chi square		38.82	1	.000
N of valid cases		100		

Sensitivity = 43/50 = 0.86 = 86%, Specificity = 38/50 = 0.76 = 76%, Odds Ratio = (43x38)/(7x12) = 19.45

Table 2: Homocysteine in smokers and non-smokers

		Smoking		
		Case	Control	Total
HCY <17	Count	6	1	7
	% with In HCY	85.7	14.3	100
	% with In GROU	21.4	4.5	14
HCY >17	Count	22	21	43
	% with In HCY	51.2	48.8	100
	% with In GROU	78.6	95.5	86.0
Total	Count	28	22	50
	% with In HCY	56.0	44	100
	% with In GROU	100	100	100
		Value	df	P value
Pearson chi square		11.79	3	0.017
N of valid cases		50		

Table 3: Homocysteine in hypertensive and non-hypertensives

		Hypertension		
		Case	Control	Total
HCY <17	Count	5	2	7
	% with In HCY	71.4	28.6	100
	% with In GROU	20.8	7.7	14
HCY >17	Count	19	24	43
	% with In HCY	44.2	55.8	100
	% with In GROU	79.2	92.3	86
Total	Count	24	26	50
	% with In HCY	48	52	100
	% with In GROU	100	100	100
		Value	df	P value
Pearson chi square		11.79	3	0.017
N of valid cases		50		

DISCUSSION

The major risk factors, along with elevated LDL cholesterol, are powerfully associated with the development of CHD. Although several of them are directly atherogenic, their power to predict CHD is still limited. Most of the excess risk for CHD can be explained by the major risk factors; this is shown by the very low risk in persons who have optimal levels of all of these risk factors. Nonetheless, when major risk factors are present, they account for only about half of the variability in CHD risk; other factors, yet to be identified, seemingly influence how much the major risk factors affect absolute CHD risk. One of that is hyperhomocysteinemia, which is studied here.

Consequently, there has been intensive research to identify new risk factors that will enhance predictive power in individuals. These newer factors can be called emerging risk factors. One of that is hyperhomocysteinemia, which is studied here.

Elevations of serum homocysteine are positively correlated with risk for CHD.¹³⁻¹⁶ The mechanism of the link between homocysteine and CHD is not well understood, although persons with inherited forms of severe homocysteinemia have premature vascular injury and atherosclerosis. In any case, the strength of association between homocysteine and CHD is not as great as that for the major risk factors. Moreover, an elevation of homocysteine is not as common as that of the major risk factor. For these reasons, ATP III does not list elevated homocysteine as a major risk factor to modify Low Density Lipoprotein-cholesterol goals.

Even though elevated homocysteine is not classified as a major risk factor, some investigators hold that the association with CHD is strong enough to make it a direct target of therapy. The available intervention for raised homocysteine was dietary folic acid, perhaps combined with other B vitamins (B6 and B12). Several clinical trials are underway to test whether homocysteine lowering will reduce CHD risk¹⁷. ATP III does not recommend routine measurement of homocysteine as part of risk assessment to modify LDL-cholesterol goals for primary prevention. This lack of recommendation is based on uncertainty about the strength of the relation between homocysteine and CHD, a lack of clinical trials showing that supplemental B vitamins will reduce risk for CHD.

Measurement of homocysteine nonetheless remains an option in selected cases, e.g., with a strong family history of premature CHD in an otherwise low-risk patient. If elevated, the clinical approach favoured by ATP III is to determine vitamin B12 level and, if this is normal, to ensure adequate folate intake rather than modifying the LDL cholesterol goal.

In in-vitro models, elevated homocysteine levels induced a hyper-coagulable state by reducing thrombomodulin level, protein C activity,¹⁸ and heparin sulphate level,¹⁹ as well as inhibiting the binding of tissue plasminogen activators to endothelial cells.²⁰ In addition to that, they activated factors V and XII,²¹ increased tissue factor expression on endothelial cells,²² and induced platelet adhesiveness and aggregation. In clinical studies, hyper homocysteinemia was associated with activation of coagulation systems in patients with premature atherosclerotic arterial disease and with thrombin generation in patients with acute coronary syndrome.

Hyperhomocysteinemia was also found to be an independent risk factor for venous thromboembolism. Furthermore, homocysteine induces expression and release of the inflammatory cytokines monocyte chemoattractant protein 1 in human monocytes and monocyte chemoattractant protein 1, vascular cell adhesion molecule 1, and interleukin 8 in endothelial cells, resulting in increased adhesion of T cells and monocytes to homocysteine-exposed endothelial cells.^{23,24} Both the prothrombotic and proinflammatory effects of elevated homocysteine levels may account for the increased risk of recurrent coronary events in patients with elevated levels of homocysteine, irrespective of the extent of the underlying coronary disease.

A number of retrospective (case-control and observational) studies done over the past 15 years indicate that homocysteine is a graded, independent risk factor for myocardial infarction, stroke, and venous thromboembolism.²⁵ In this study homocysteine levels were elevated and statistically highly significant.

Prospective studies, in contrast, were revealing both positive and negative associations.^{26,27} The Tromsø study, one of the longitudinally followed cohorts, revealed a 40% increase in the risk of myocardial infarction associated with a 4 mmol/L increase in

tHcy, although reanalysis of the Physicians' Health Study data and initial findings of the Atherosclerosis Risk In Communities trial revealed no such associations. In the current study, the association of high tHcy with age were not established. These results are concordant with the results from the study by Nguyen et al.

A study on lipoprotein (a): better assessor of coronary heart disease risk in south Indian population by D. Rajasekhar et al shows Low levels of total-C and High Density Lipoprotein-Cholesterol observed in patients when compared to controls. Low levels of HDL-C are reported to increase the risk of CHD even when total cholesterol is not elevated. But in this present study, HDL-C levels are not correlated with CHD. Increased total-C and LDL-C levels are reported in patients than in controls. In this study, high levels of LDL-C was observed in patients against controls.

A meta-analysis²⁸ found that for every 2.5- $\mu\text{mol/L}$ increase in plasma total homocysteine, the risk of myocardial infarction increases by about 10% and the risk of stroke increases by about 20%. The relationship between homocysteine and risk appears to hold for plasma concentrations of total plasma concentrations of total homocysteine between 10 and 30 $\mu\text{mol/L}$.

For almost two decades we have known that a high plasma homocysteine level (hyperhomocysteinemia) is associated with increased cardiovascular risk. But whether elevated homocysteine causes cardiovascular disease or is a consequence of it remains unknown. Adding folic acid and other B vitamins to the diet is effective in lowering levels, but whether it improves the clinical outlook is also still uncertain and is the focus of several ongoing clinical trials. On the other hand, there is good reason to believe that homocysteine is an independent cardiovascular risk factor and that homocysteine lowering therapy ultimately may prove to have a modest clinical benefit. Treating with either folic acid 0.4–5.0 mg/day or vitamin B12 0.5–1.0 mg/day or both are inexpensive and presumably safe, and is likely to be cost effective for preventing cardiovascular events if ongoing clinical trials confirm that homocysteine lowering therapy is beneficial. Even without definitive evidence of clinical benefit, a case can be made for detecting and treating hyper

homocysteinemia in selected patients (ie, those with a history of premature cardiovascular disease, stroke, or venous thromboembolism, or those thought to be at high risk because other risk factors are present). In such cases, treatment is unlikely to cause harm and may produce an important benefit. In this study the association of hyperhomocysteinemia with other emerging risk factors, like lipoprotein A and hs-CRP were not analyzed. This is the limitation of this study.

CONCLUSION

The association of hyperhomocysteinemia with CHD was significant. Homocysteine values were higher in smokers and hypertensives. Established risk factors like hypertension, diabetes, LDL-C, family history of CHD were higher in cases than controls. Smoking and decreased HDL-C were not significantly associated with CHD.

ACKNOWLEDGEMENT

The authors are grateful to the Dean, the Head Of the Department, General Medicine and Biochemistry of our institution Meenakshi medical college hospital and research institution, Kanchipuram for providing facilities for research.

Financial support: Nil.

Conflict of Interest: Nil.

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